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Regulation of uncoupling proteins 2 and 3 in porcine adipose tissue☆

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Abstract

This study was performed to determine whether or not uncoupling protein 2 (UCP2) and UCP3 expression in porcine subcutaneous adipose tissue are hormonally regulated in vitro and whether their expression is correlated with changes in metabolic activity. Tissue slices (approximately 100 mg) were placed in 12-well plates containing 1 mL of DMEM/F12 with 25 mM Hepes, 0.5% BSA, pH 7.4. Triplicate slices were incubated with basal medium or hormone supplemented media at 37 °C with 95% air/5% CO_2. Parallel cultures were maintained for either 2 or 24 h to evaluate metabolic viability of the tissue. Slices were transferred to test tubes containing 1 mL of DMEM/F12 with 25 mM Hepes, 3% BSA, 5.5 mM glucose, 1 μCi ^{14}C-U-glucose/mL and incubated for an additional 2 h at 37 °C. Glucose metabolism in 2-h incubations did not differ from 24-h (chronic) incubations, indicating viability was maintained ($P > 0.05$). Expression of UCP2 and UCP3 was assessed in slices following 24 h of incubation with various combinations of hormones by semi-quantitative RT-PCR. Expression of UCP2 was induced by leptin (100 ng/mL; $P < 0.05$). Growth hormone (100 ng/mL) inhibited UCP2 expression ($P < 0.05$). Expression of UCP3 was inhibited by growth hormone (100 ng/mL; $P < 0.05$), triiodothyronine (10 nM; $P < 0.05$) or leptin (100 ng/mL; $P < 0.05$). Changes in UCP expression could not be associated with overall changes in glucose metabolism by adipose tissue slices in chronic culture. Published by Elsevier Inc.

Keywords: Uncoupling protein 2; Uncoupling protein 3; Adipose tissue; Swine

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1. Introduction

The presence of an uncoupling protein was first detected in brown adipose tissue of hibernating animals and believed accountable for non-shivering thermogenesis [1]. This protein, uncoupling protein 1 (UCP1), was demonstrated to mediate ATPase independent proton leakage at the inner mitochondrial membrane [2]. UCP1 dissipates the transmembrane electrochemical potential by transporting protons from the intermembrane space back toward the matrix of the mitochondria, promoting a proton leakage and generating energy. The consequence of UCP1 activity is heat production through uncoupling ATP formation from cellular respiration. However, proton leakage exceeds all that can be accounted for by UCP1 activity, implicating additional proteins and leading to the discovery of additional uncoupling proteins.

UCP2 and UCP3 share 55% and 57% amino acid identity with UCP1 and 73% with each other [3,4]. UCP2 mRNA has been shown to be expressed in a range of tissues, including adipose tissue and skeletal muscle [3]. The actual UCP2 protein has been confirmed to be present in adipose tissue by Western analysis, but not present in skeletal muscle [5]. Feeding a high fat diet in rodents produces an increase in the expression of UCP2 in adipose tissue [3,6]. This response by UCP2 has been proposed to result in an increase in the formation of reducing equivalents necessary for lipogenesis [7].

Uncoupling protein 3 is expressed primarily in skeletal muscle in rodents, with limited expression in adipose tissue [8]. Using UCP3 knockout mice, UCP3 has been associated with changes in mitochondrial energy production that suggests uncoupling activity specific to skeletal muscle [9]. The combined results of the research into the potential functions of UCP2 and UCP3 may suggest a function for them in tissue specific energy metabolism/expenditure.

The porcine UCP2 and UCP3 sequences have been cloned [10]. The pig UCP2 and UCP3 sequences are 89% and 88% homologous, respectively, to the human homologues. Comparison of the pig UCP2 and UCP3 sequences reveals them to be 76% identical. Damon et al. [11] have reported that both UCP2 and UCP3 are expressed in porcine skeletal muscle and adipose tissue. However, the regulatory mechanisms for UCP2 or UCP3 expression have not been examined in swine adipose tissue. The present study was performed to determine whether or not UCP2 and UCP3 expression in porcine adipose tissue is hormonally regulated.

To accomplish this goal requires an in vitro system for chronic incubation ($\geq$24 h) of porcine adipose tissue. Previous studies have been unable to produce a chronic incubation system wherein the metabolic rate has not declined with time [12,13]. Therefore, the second objective of this experiment was to develop a chronic incubation system for adipose tissue slices and to determine if changes in tissue metabolism are related to changes in UCP2 or UCP3 expression.

2. Material and methods

An acute regulation of UCP2 or UCP3 expression has not been demonstrated in adipose tissue. These proteins appear to respond primarily to more chronic stimuli. Therefore, a

chronic adipose tissue incubation system had to be developed to permit examination of the hormonal regulation of UCP2 and UCP3.

Briefly, adipose tissue was collected from 75 to 80 kg barrows (Yorkshire × Landrace; $n = 5$). Dorsal subcutaneous adipose tissue samples from between the second and fourth thoracic vertebrae were acquired following euthanasia by electrical stunning and exsanguination according to procedures approved by the Institutional Area Animal Use and Care Committee. Dissected adipose tissue was diced into 1 cm × 4 cm strips and placed in Hanks buffer (37 °C; pH 7.4) in screw capped polypropylene Erlenmeyer flasks for transport to the laboratory, approximately 2 min from the abattoir.

In the laboratory, adipose tissue strips were placed in fresh Hanks buffer (37 °C; pH 7.4). Adipose tissue strips were dissected clean of any extraneous muscle tissue and further separated into 1 cm cubes in a laminar flow hood. Adipose tissue explants (approximately 100 mg) were prepared by slicing tissue cubes with a Stadie–Riggs microtome. Tissue slices (400 μm thickness) were rinsed twice with fresh Hanks buffer (37 °C; pH 7.4), blotted free of excess liquid and weighed. Tissue slices were then transferred to 12-well tissue culture plates containing 1 mL of DMEM/F12 with 25 mM Hepes, 0.5% BSA, pH 7.4 and the various hormone supplements of interest. Triplicate tissue slices were incubated with either basal medium or hormone supplemented media in a tissue culture incubator at 37 °C with 95% air/5% CO_2.

Hormones included 100 nM insulin, 100 ng/mL growth hormone, 1 μM dexamethasone, 10 nM tri-iodothyronine, or 100 ng/mL recombinant porcine leptin. Sterile hormone solutions were prepared and frozen in vials prior to initiation of the experiment. Insulin (Sigma–Aldrich, St. Louis, MO) was solubilized in 0.001N HCl. Dexamethasone was solubilized in ethanol. Porcine growth hormone (USDA-pGH-B-1, Beltsville, MD) was diluted in 25 mM sodium bicarbonate buffer, pH 9.4 (Na_2CO_3, $NaHCO_3$). Tri-iodothyronine (T_3) was prepared in 0.01N NaOH. Recombinant porcine leptin [14] was solubilized with PBS. Following initial solubilization, all hormones were subsequently diluted in 0.5% BSA in saline, aliquoted and frozen. Individual hormone aliquots were thawed for each day of use and diluted in incubation medium to the appropriate concentration. The selected hormone concentrations were determined in preliminary experiments or based upon concentrations described in the literature to affect porcine adipose tissue metabolism or uncoupling protein expression [15–18].

Parallel cultures were maintained for either 2 or 24 h to evaluate metabolic viability. At the end of the respective incubation period, tissue samples from these incubations were blotted and transferred to polypropylene 16 × 100 test tubes (Sarstedt) containing 1 mL of incubation medium. Glucose incubation medium was comprised of DMEM/F12, 25 mM Hepes, 3% BSA, 5.5 mM glucose and 1 μCi ^{14}C-U-glucose/mL (Moravek Biochemicals, Brea, CA).

Following addition of the tissue slices, tubes were gassed with 95% air/5% carbon dioxide and then capped with rubber stoppers containing center wells. Tubes were then incubated for 2 h at 37 °C in a shaking water bath (90 oscillations/min). All treatments were performed in triplicate and the experiment was repeated with adipose tissue slices isolated from five different animals.

Following 2 h in the shaking water bath, 0.5 mL 1N H_2SO_4 was injected into the medium to kill the metabolic activity of the cells. Ten minutes later, 250 μL of methylbenzythonium

hydroxide (Sigma, St. Louis, MO) was injected into the center wells. Carbon dioxide was captured during a 30-min incubation. Stoppers were then removed and the center wells were transferred to scintillation vials for counting. The tissue slices were transferred to 16 × 125 screw capped test tubes. Five milliliters of Dole's solution was added to incubation tubes, vortexed and transferred to the test tubes. Lipid extractions were performed according to the method of deCingolani [19]. Incorporation of label into CO_2, total lipid and fatty acids following saponification was determined as described by Azain and Martin [20].

2.1. Gene expression analyses

The expression of UCP2 and UCP3 was assessed following 24 h of incubation in various combinations of the hormone preparations described above by real time PCR analysis. Tissue samples from these incubations were blotted and transferred to microfuge tubes with subsequent freezing in liquid nitrogen and storage at −80 °C prior to analysis for uncoupling protein gene expression.

Total RNA was isolated using TRI reagent according to the manufacturer's protocol (Sigma–Aldrich, St. Louis, MO). RNA integrity was assessed via agarose gel electrophoresis and RNA concentration and purity were determined spectrophotometrically using A260 and A280 measurements.

Reverse transcription (RT) and real-time PCR analysis were performed in a single tube using the QuantiTect SYBR Green RT-PCR protocol (Qiagen Inc., Valencia, CA). Reactions (25 μL) consisted of 1 μg total RNA, 12.5 μL QuantiTect SYBR Green RT-PCR Master Mix, 0.5 μM primers, 0.25 μL QuantiTect RT Mix and 9 μL RNase-free H_2O. Thermal cycling and data acquisition were performed with a DNA Engine Opticon Continuous Fluorescence Detection System (MJ Research, Waltham, MA). Thermal cycling parameters were as follows: 1 cycle 50 °C for 30 min (reverse transcription), 1 cycle 95 °C for 15 min (PCR activation), followed by 30 cycles, 94 °C for 15 s, 58 °C for 30 s, 72 °C for 30 s, with a final extension at 72 °C for 8 min. Melting curve analysis was performed on all RT-PCR reactions to confirm specificity and identity of the RT-PCR products. Specificity of RT-PCR products was further confirmed by agarose gel electrophoresis. The RT-PCR techniques were optimized in a series of preliminary experiments. The one-step RT-PCR for UCP2, UCP3 and 18S was optimized for linearity (exponential amplification) from >20 to <30 cycles under the conditions described above.

The following primers were used for generating 210-base pair fragments corresponding to a part of the translated UCP2 sequence: 5′-CTGCAGATCCAGGGAGAAAG-3′ (forward), 5′-GCTTGACGGAGTCGTAGAGG-3′ (reverse). The primers for UCP3 were used to generate 200 base pair fragments corresponding to a part of the translated sequence: 5′-ACGATGGATGCCTACAGGAC-3′ (forward), 5′-TCCGAAGGCAGAGACAAAGT-3′ (reverse). The primers for 18S ribosomal RNA were purchased (QuantumRNA™ Universal 18S Internal Standard, Ambion Inc., Austin, TX). The UCP2 and UCP3 amplicons were excised from an agarose gel, re-amplified, and run through a GenElute PCR clean-up kit (Sigma Chemical Co., St. Louis, MO). UCP2 and UCP3 amplicons were sequenced to confirm identity using automated fluorescent DNA sequencing (ABI 310, Perkin-Elmer Applied Biosystems, Foster City, CA).

2.2. Quantification of gene expression

At the end of the PCR, baseline and threshold crossing values (C_T) for UCP2, UCP3 and 18S were calculated using the Opticon Monitor Software (Version 1.06; MJ Research, Waltham, MA) and the C_T values were exported to Microsoft Excel for analysis. The relative expression of UCP2 and UCP3 mRNA were calculated using the comparative C_T method according to manufacturer's literature (MJ Research). The C_T values were transformed to their respective antilogarithmic values. The proportionality of C_T values for UCP2, UCP3 and 18S relative to total RNA are graphically presented in Fig. 1. The relative amount of UCP2 or UCP3 mRNA, standardized against the amount of 18S mRNA, in adipose tissue explants was expressed as $\Delta C_T = [C_T\text{UCP} - C_T\text{18S}]$. The ratio of UCP mRNA/18S mRNA, i.e., the relative UCP expression, was then calculated as 2^{-C_T}. Data are presented as the % change in amplicon number relative to the control group amplicon number for each gene. Values are presented as the mean ± S.E.M. of determinations from 5 to 6 individual animals.

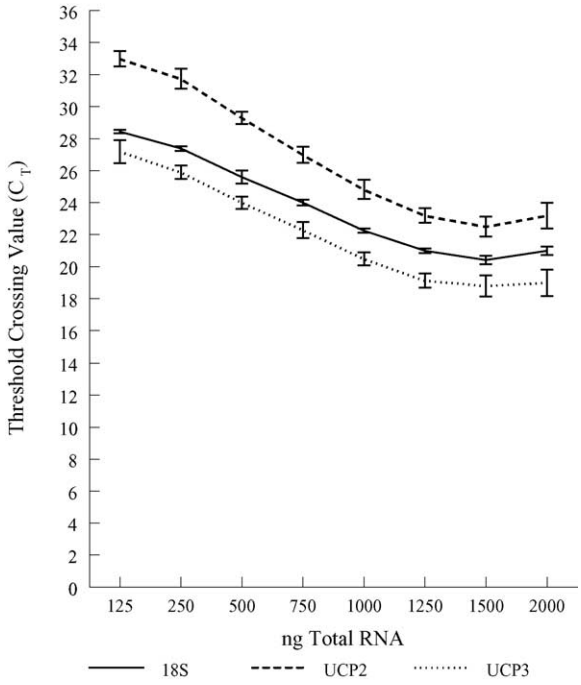

Fig. 1. Changes in C_T with increasing concentrations of total RNA. Total RNA was extracted from porcine subcutaneous adipose tissue and diluted in a series of tubes from 2000 ng/tube to 125 ng/tube. Five replicate tubes were used at each concentration of RNA for reverse transcription and real time PCR analysis for UCP2, UCP3 and 18S as described in the Methodology. Data acquisition was performed using the Opticon Monitor software (version 1.06) provided with the DNA Engine Opticon Continuous Fluorescence Detection System. Threshold crossing values (C_T) were calculated by the software and are plotted relative to the ng of total RNA in the original reverse transcription reactions.

2.3. Statistical analysis

The experimental model for these experiments was a completely randomized design. Blocking was accomplished by converting data to percentages, relative to basal medium to account for culture-to-culture variation. Data were analyzed by one-way analysis of variance using SigmaStat software (SPSS Science, Chicago, IL). Mean separation was analyzed using Student–Newman–Keuls test. Means were defined as significantly different at $P < 0.05$.

3. Results

Glucose oxidation, conversion to total lipids and specific incorporation into fatty acids were evaluated following 2 and 24 h of tissue incubation in the presence of a variety of hormones to evaluate the feasibility of using chronic tissue slice incubations to measure changes in adipose tissue gene expression. No difference in glucose oxidation was detected between cultures incubated for 2 or 24 h in basal medium (Fig. 2; $P > 0.05$). Insulin (100 nM) addition to the medium produced similar increases in glucose oxidation relative to basal medium for 2 and 24 h incubations ($P < 0.05$). Acute incubation with dexamethasone (1 μM) had no effect on glucose oxidation ($P > 0.05$). Chronic incubation with dexamethasone resulted in a 70% reduction in the oxidation rate relative to basal medium ($P < 0.05$). The combination of insulin and dexamethasone (ID) produced similar increases (∼120%) in glucose oxidation for both acute and chronic incubations relative to basal medium ($P < 0.05$). Both acute and chronic incubation with insulin, dexamethasone and T_3 (IDT, 10 nM) produced similar increases in glucose oxidation relative to basal medium ($P < 0.05$). The rate of glucose oxidation in response to leptin (100 ng/mL) addition to medium containing insulin and dexamethasone (IDL) was not different between acute and chronic incubations ($P > 0.05$); however chronic treatment with IDL reduced the glucose oxidation rate sufficiently that it did not differ from the rate in basal medium ($P > 0.05$).

Glucose incorporation into fatty acids was unaffected by time of incubation in basal medium (Fig. 3; $P > 0.05$). Insulin addition to the medium produced a doubling in the lipogenic rate in both acute and chronic incubations ($P < 0.05$). Dexamethasone produced a 27% increase in glucose incorporation into fatty acids in acute incubations ($P < 0.05$). However, chronic incubation with dexamethasone produced an 88% inhibition of fatty acid synthesis ($P < 0.05$). The addition of both insulin and dexamethasone to the medium produced similar increases in lipogenesis in both acute and chronic incubations ($P < 0.05$). Medium containing IDT produced a similar lipogenic response as ID medium ($P > 0.05$), with no difference between acute and chronic incubations with IDT ($P > 0.05$). Acute incubation with IDL did not alter the lipogenic rate of acute incubations in comparison to ID ($P > 0.05$). However, addition of leptin to chronic incubations with ID produced a 30% inhibition of fatty acid synthesis ($P < 0.05$; acute versus chronic), but still a greater rate than basal incubations ($P < 0.05$; IDL versus basal medium). Measurements of total lipid synthesis were also performed for this experiment and the data completely paralleled the responses in fatty acid synthesis; therefore, total lipid synthesis data are not presented.

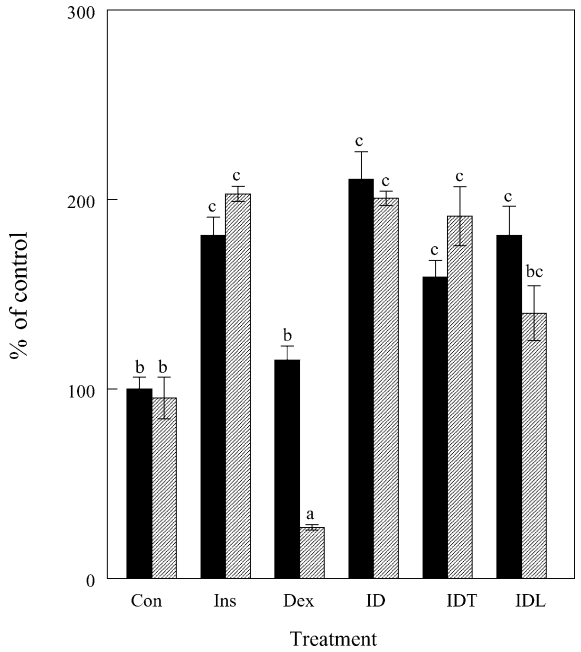

Fig. 2. Relative glucose oxidation in response to acute vs. chronic hormone exposure. Adipose tissue sections (100 mg) were incubated in a basal medium ± porcine insulin (100 nM); ±dexamethasone (1 μM) or combinations of these hormones ± T_3 (10 nM) ± leptin (100 ng/mL) for 24 h, followed by incubation in a medium containing 1 μCi ^{14}C-U-glucose/mL for 2 h, followed by collection of $^{14}CO_2$ for analysis of glucose oxidation. Data are expressed relative to cultures incubated without hormone additions. Black bars represent acute 2 h incubations, while gray bars represent 24 h incubations. Values not sharing a common superscript letter are different ($P < 0.05$; $n = 5$). Con = control (basal medium), Ins = 100 nM insulin, Dex = 1 μM dexamethasone, ID = 100 nM insulin + 1 μM dexamethasone, IDT = 100 nM insulin + 1 μM dexamethasone + 10 nM T_3 and IDL = 100 nM insulin + 1 μM dexamethasone + 100 ng/mL porcine leptin.

Incubation of adipose tissue slices for 24 h with insulin (100 nM) did not change the level of UCP2 or UCP3 expression relative to tissue incubated with basal medium (Fig. 4; $P > 0.05$). Dexamethasone (1 μM) elevated UCP2 expression, but not significantly ($P > 0.05$). While UCP3 was also elevated by dexamethasone treatment, the effect was not statistically significant due to variability in response among pigs ($P > 0.05$). Similarly, the combination of insulin and dexamethasone increased UCP2 and UCP3 mRNA levels in comparison to basal medium, but the variability in response precluded detection of a statistically significant effect ($P > 0.05$).

Expression of UCP2 was elevated by T3 or T3 in combination with insulin and dexamethasone, but was not statistically significant (Fig. 5; $P > 0.05$). In contrast, T3 inhibited UCP3 expression ($P < 0.05$). Uncoupling protein 3 was also inhibited by T3 in combination with insulin and dexamethasone ($P < 0.05$).

Leptin (100 ng/mL) stimulated UCP2 expression (Fig. 6; $P < 0.05$). However, leptin inhibited UCP3 expression ($P < 0.05$). Addition of leptin to medium containing insulin and dexamethasone did not significantly affect UCP2 expression ($P > 0.05$). However, this

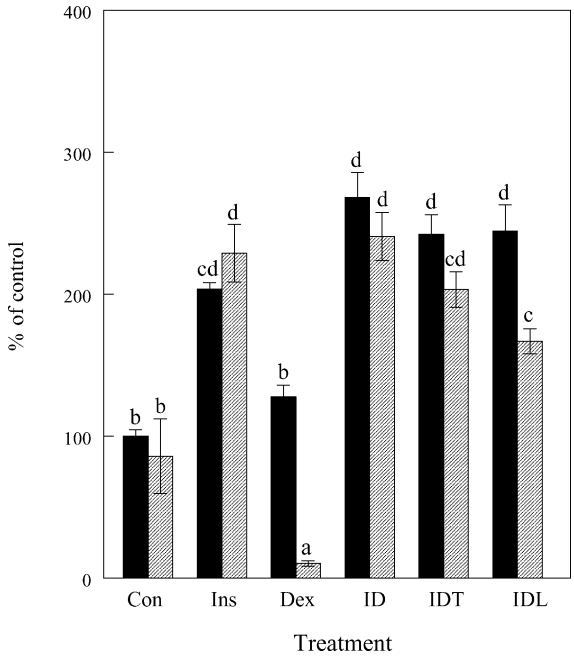

Fig. 3. Relative glucose incorporation into fatty acids in response to acute vs. chronic hormone exposure. Adipose tissue sections (100 mg) were incubated in a basal medium ± porcine insulin (100 nM); ±dexamethasone (1 μM) or combinations of these hormones ± T_3 (10 nM) ± leptin (100 ng/mL) for 24 h, followed by incubation in a medium containing 1 μCi ^{14}C-U-glucose/mL for 2 h, followed by extraction of fatty acids as described in Section 2. Data are expressed relative to cultures incubated without hormone additions. Black bars represent acute 2 h incubations, while gray bars represent 24 h incubations. Values not sharing a common superscript letter are different ($P < 0.05$; $n = 5$). Con = control (basal medium), Ins = 100 nM insulin, Dex = 1 μM dexamethasone, ID = 100 nM insulin + 1 μM dexamethasone, IDT = 100 nM insulin + 1 μM dexamethasone + 10 nM T3 and IDL = 100 nM insulin + 1 μM dexamethasone + 100 ng/mL porcine leptin.

hormonal combination suppressed the expression of UCP3 to a similar extent as leptin alone ($P < 0.05$).

Porcine GH (100 ng/mL) inhibited UCP2 and UCP3 expression relative to basal medium, (Fig. 7; $P < 0.05$). Porcine GH in combination in combination with insulin (100 nM) and dexamethasone (1 uM) inhibited UCP3 to a similar degree as GH alone ($P < 0.05$). However, porcine GH in combination with insulin and dexamethasone increased UCP2 mRNA levels in comparison to basal medium, but the variability in response precluded detection of a statistically significant effect ($P > 0.05$).

4. Discussion

The use of isolated adipocytes to monitor gene expression is the ideal system, as it removes the confounding influence of stromal vascular cells and more uniform incubations can be performed because known amounts of adipocytes can be added to each incubation.

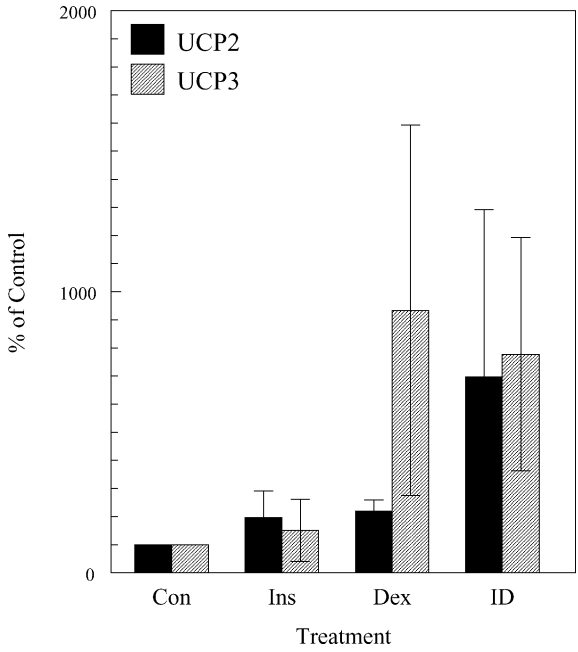

Fig. 4. Relative UCP2 and UCP3 expression in response to chronic hormone exposure. Adipose tissue sections (100 mg) were incubated in a basal medium ± porcine insulin (100 nM); ±dexamethasone (1 μM) or combinations of these hormones, followed by extraction of total RNA. Total RNA (1 μg) was used in RT-PCR with data acquisition performed using a DNA Engine Opticon Continuous Fluorescence Detection System. Data are corrected using an 18S rRNA as a reference value and then data are expressed as a percentage relative to tissue incubated without hormone additions. No significant differences. Con = control (basal medium), Ins = 100 nM insulin, Dex = 1 μM dexamethasone and ID = 100 nM insulin + 1 μM dexamethasone.

However, chronic incubation (24 h or more) of adipocytes is fraught with difficulties due to the fragility of the isolated adipocyte [21]. The technique for using adipose tissue fragments in chronic incubations has matured during the past 10 years and has developed into a method of choice for examining chronic metabolic responses as well as chronic adaptations in gene expression [21–24]. This technique has been previously used with porcine adipose tissue fragments to examine the metabolic response to insulin and growth hormone [12,13]. However, the chronic responses decayed with time, suggesting a suboptimal system. The present study used a modification of this technique and took advantage of methods developed with recent human adipose tissue culture systems [21].

The data in the present study clearly demonstrate that the metabolic status of porcine adipose tissue is unchanged in the basal state following 24 h of incubation, when compared to a freshly prepared 2 h incubation derived from the same tissue sample. Neither glucose oxidation total lipid synthesis nor fatty acid synthesis was deleteriously affected by chronic incubation. Addition of insulin to the medium stimulated glucose metabolism in the range of 70–130% across the metabolic parameters monitored. This is comparable to the highest rates measured for swine adipose tissue in vitro [12,25].

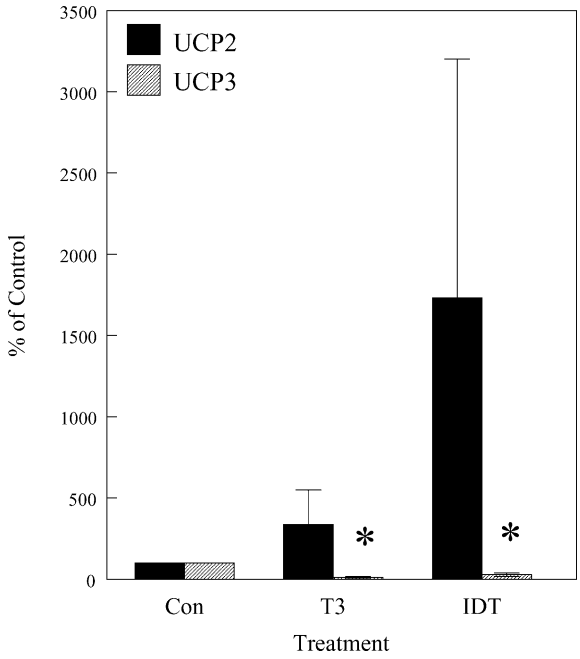

Fig. 5. Relative UCP2 and UCP3 expression in response to chronic hormone exposure. Adipose tissue sections (100 mg) were incubated in a basal medium ± T_3 (10 nM) (porcine insulin (100 nM); +dexamethasone (1 μM), followed by extraction of total RNA. Total RNA (1 μg) was used in RT-PCR with data acquisition performed using a DNA Engine Opticon Continuous Fluorescence Detection System. Data are corrected using an 18S rRNA as a reference value and then data are expressed as a percentage relative to tissue incubated without hormone additions. (*) Significantly different from control ($P < 0.05$; $n = 4$). Con = control (basal medium), T3 = 10 nM tri-iodothyronine and IDT = 100 nM insulin + 1 μM dexamethasone + 10 nM tri-iodothyronine.

Dexamethasone did not affect glucose metabolism of acute incubations. However, chronic dexamethasone treatment produced a severe inhibition across all parameters of glucose metabolism monitored. This inhibition did not reflect a loss of cell viability as isolation of total RNA with intact 28S and 18S rRNA was possible. Secondly, UCP2 and UCP3 mRNA levels were stable and similar to levels present in insulin treated cultures. Rather the inhibition of glucose metabolism in chronic incubations may be due to development of dexamethasone-induced inhibition of glucose transport within the adipocyte [26–28].

Hajduch et al. [29] have reported that insulin combined with dexamethasone synergistically increase GLUT4 in rat adipocytes, resulting in elevated glucose metabolism. However, no synergism of the combined hormones was detected for the monitored metabolic parameters in the present study. Synergism of insulin and glucocorticoids has previously been reported during the differentiation of swine preadipocytes [17]; this may be due to mature pig adipocytes not reflecting the metabolic activity of newly formed adipocytes in vitro or an age related shift in sensitivity to these hormones in swine adipocytes.

Tri-iodothyronine did not affect glucose metabolism of the porcine adipocyte when used in combination with insulin and dexamethasone. Studies in other species indicate that T_3

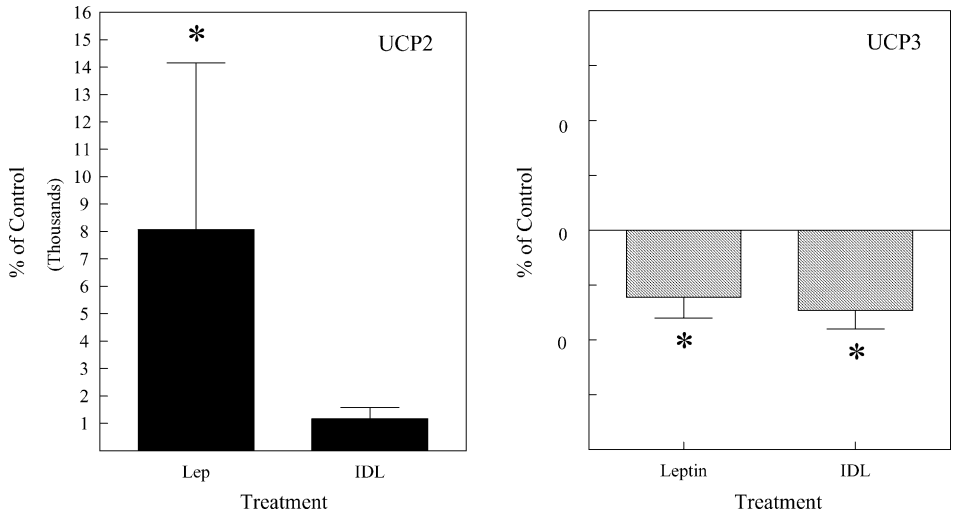

Fig. 6. Relative UCP2 and UCP3 expression in response to chronic hormone exposure. Adipose tissue sections (100 mg) were incubated in a basal medium ± porcine leptin (100 ng/mL) (porcine insulin (100 nM); +dexamethasone (1 μM), followed by extraction of total RNA. Total RNA (1 μg) was used in RT-PCR with data acquisition performed using a DNA Engine Opticon Continuous Fluorescence Detection System. Data are corrected using an 18S rRNA as a reference value and then data are expressed as a percentage relative to tissue incubated without hormone additions. (*) Significantly different from control ($P < 0.05$; $n = 4$). Con = control (basal medium), Lep = 100 ng/mL recombinant porcine leptin and IDL = 100 nM insulin + 1 μM dexamethasone + 100 ng/mL recombinant porcine leptin.

can inhibit glucose transport in the rodent adipocyte [30,31]. Leptin has been previously demonstrated to inhibit glucose metabolism in porcine adipose tissue [16]. The present study confirms these previous results and provides further evidence for a role of leptin in partitioning energy away from lipid accretion in the pig adipocyte, for use by other tissues.

The results of this study demonstrate that this tissue culture system maintains adipose tissue viability for at least 24 h and is useful for examination of metabolism in response to chronic hormone treatment and thus may be a useful system to examine changes in the regulation of gene expression over time. Regulation of the expression of uncoupling proteins has not been clearly defined, primarily due to the absence of in vitro studies wherein the cellular environment can be controlled. The present study has confirmed the presence of UCP2 and UCP3 mRNA in porcine adipose tissue, as previously demonstrated by Damon et al. [11]. This study has added new information demonstrating that pig UCPs can be hormonally regulated, implying endocrine regulation may occur in vivo.

Changes in insulin status have been correlated with changes in adipose UCP2 and UCP3 expression [32], however, a direct role for insulin in regulation of UCP2 or UCP3 expression within adipose tissue has not been demonstrated for any species. The present study could not confirm a role for insulin in the regulation of UCP expression in porcine adipose tissue, despite insulin's significant effects on glucose metabolism.

Chronic dexamethasone treatment significantly suppressed adipocyte glucose metabolism yet had no statistically significant effect on UCP2 or UCP3 expression. The only

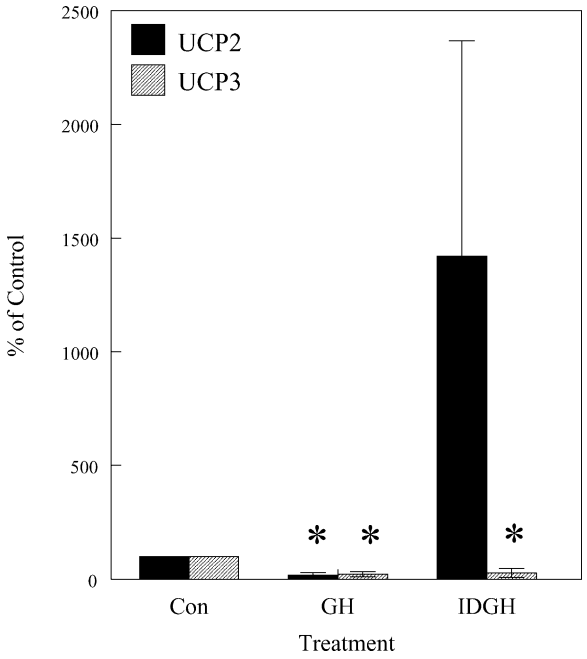

Fig. 7. Relative UCP2 and UCP3 expression in response to chronic hormone exposure. Adipose tissue sections (100 mg) were incubated in a basal medium ± porcine growth hormone (100 ng/mL) ± porcine insulin (100 nM); +dexamethasone (1 μM), followed by extraction of total RNA. Total RNA (1 μg) was used in RT-PCR with data acquisition performed using a DNA Engine Opticon Continuous Fluorescence Detection System. Data are corrected using an 18S rRNA as a reference value and then data are expressed as a percentage relative to tissue incubated without hormone additions. (*) Significantly different from control ($P < 0.05$; $n = 4$). Con = control (basal medium), GH = 100 ng/mL porcine growth hormone and IDGH = 100 nM insulin + 1 μM dexamethasone + 100 ng/mL porcine growth hormone.

report examining the potential function of glucocorticoids in regulating UCP2 or UCP3 could not demonstrate an effect of dexamethasone on UCP2 expression in differentiating rat adipocyte cultures [33]. The expression of UCP3 was highly variable in response to dexamethasone or the combination of insulin with dexamethasone. Expression of UCP2 was also extremely variable in response to the combination of insulin and dexamethasone. This variability was sufficient to mask detection of any significant differences in UCP2 or UCP3 expression relative to control incubations. The causative factors for this variability are unknown.

Uncoupling protein 3 expression was shown in the present study to be inhibited in response to T_3 treatment. These data are in contrast with the reports in skeletal muscle of rats by Larkin et al. [34] and in L6 myoblasts by Nagase et al. [35]. Masaki et al. [15] have reported that UCP2 in adipose tissue is induced by in vivo T_3 treatment, but not UCP3. Again this may be the result of T_3's overall effects on peripheral metabolism, rather than a specific effect on adipose tissue, as the present study could not demonstrate this UCP3 response in pig adipose tissue slices. No relationship could be made between T_3 treatment and UCP3 in the present study with any changes in the parameters of glucose metabolism

that were measured. Fatty acid oxidation in skeletal muscle has been associated with UCP3 expression and a role for UCP3 in regulating fatty acid oxidation has been proposed [2,36]. However, porcine adipose tissue oxidizes less than 1–2% of the total fatty acids metabolized with >98% esterified [37]. Thus, a role for UCP3 in fatty acid oxidation in porcine adipose tissue is probably irrelevant.

Leptin was shown in the present study to inhibit lipogenesis, while stimulating UCP2 and inhibiting UCP3 expression in chronic incubations of porcine adipose tissue slices. Ceddia et al. [38] reported that leptin stimulates the expression of UCP2, but did not affect UCP3 expression in adipocytes from rodents, while producing similar effects on metabolism as in the present study. The concentration of leptin used was the same; therefore, comparison of the data between studies suggests some species specificity in the response of the UCP3 gene to leptin. The data suggest that a decline in UCP3 expression is associated with a leptin-induced inhibition of glucose metabolism in swine adipose tissue; however, further studies will be necessary to determine the extent of this association.

The inhibition of UCP2 by growth hormone has not been reported for any species. Whether this relative reduction in UCP2 mRNA expression is associated with a shift in energy expenditure by the adipocyte is unknown. The decrease in UCP2 mRNA observed in the present study could not be associated with any parameters of adipose tissue metabolism in the present study, as GH was not assessed for its effects on metabolism. Previous studies have demonstrated that GH inhibits glucose oxidation and lipogensis in vitro [12,13]. However, this does not preclude the change in UCP2 expression from being correlated with lipolysis or other metabolic parameters in the adipocyte. The combination of insulin and dexamethasone could overcome the GH-induced UCP2 inhibition. This is surprising as glucocorticoids have been demonstrated to potentiate the action of GH on pig adipose tissue metabolism [13]; suggesting that GH inhibition of UCP2 expression may not be associated with adipose metabolism. Uncoupling protein 3 gene expression has been demonstrated to double in response to GH treatment in GH deficient subjects [39]. Yet in the present study a decrease in UCP3 level was detected with GH treatment of adipose tissue in vitro. The numerous actions of GH on peripheral tissues and its ability to shift overall energy partitioning raise the question of whether the human in vivo UCP3 response is a direct function of GH. The data do imply that UCP3 expression is associated with a GH-induced inhibition of glucose metabolism in swine adipose tissue.

The present study has demonstrated that expression of the genes for UCP2 and UCP3 are hormonally regulated in porcine adipose tissue. Whether changes in expression are the consequence of changes in the rate of transcription or in rates of mRNA degradation are unknown. The regulation of these genes cannot be directly associated with changes in the metabolic activity of subcutaneous adipose tissue. In skeletal muscle, changes in UCP3 content accompany changes in glucose metabolism with type 2 diabetes [40]. In adipose tissue from the present study, leptin-induced inhibition of glucose metabolism was associated with a decrease in UCP3 expression, while dexamethasone-induced inhibition of glucose metabolism was not associated with any change in UCP3 expression. Conversely, insulin stimulation of glucose metabolism was not associated with any changes in UCP2 or UCP3 expression. These data imply a primary response to the hormonal stimuli and at best a peripheral association with changes in glucose metabolism and lipogenesis. However, the present study cannot exclude a potential role of UCP2 and UCP3 in overall porcine

adipose tissue metabolism. The uncoupling proteins may have a role in lipolysis. Previous studies in rodents have demonstrated an association between skeletal muscle UCP2 [41] and UCP3 [42] and changes in free fatty acid levels with fasting; however, there has not been a direct examination of the relationship of adipose lipolysis and UCP expression. In addition, further studies will be required to more directly examine mitochondrial energy expenditure in response to hormonal stimuli to determine whether UCP2 and UCP3 have a role in energy expenditure in porcine adipose tissue.

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